bio-protocol Preprint

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Glucose (2-NBDG) uptake assay

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An abbreviated version of this protocol was published in Cancer & Metabolism in Apr 2021

Tumor suppressor SMAR1 regulates PKM alternative splicing by HDAC6-mediated deacetylation of PTBP1

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Detailed protocol

Glucose (2-NBDG) uptake assay:

Transfection:

- Seed 1 x 10^6 MCF7 cells/ per well in 6 well plate in DMEM with 10% FBS and incubate at 37C for 24 hours in 5% CO_2 incubator.
- MCF7 cells were transfected with SMAR1 siRNA and control siRNA by lipofectamine RNAiMax™ (Ambion) according to the manufacturer's protocol. Amount of siRNA and lipofectamine RNAiMax™ are used as mentioned in the table:
    1. ul

siRNA per well	25 pmol
lipofectamine RNAiMax™	7.5 ul

2-NBDG treatment followed by FACS analysis:

- After 24 h of transfection, media was removed and replenished with 10% FBS containing DMEM (without glucose and sodium pyruvate) and incubated at 37 °C for 1 h.
- 10 μM fluorescent d-glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-d-glucose (2-NBDG) (Invitrogen) was added to culture media and cells were incubated for 1 h at 37 °C.
- The 2-NBDG uptake reaction was stopped by removing the incubation medium and the cells were washed with ice-cold 1× PBS.
- Cells were harvested by trypsinization followed by washing with ice cold 1x PBS. (Repeat the washing twice).
- Cells were divided in two tubes and centrifuged at 2000 RPM for 3 mins at 4C.
- One tube was used for western blot to confirm the knockdown.
- Remaining tube was further resuspended in 500 ul of ice cold 1x PBS.
- 1 μg/ml propidium iodide (PI) was added to distinguish the viable cell population before the FACS analysis.
- For each measurement, data from 10,000 single-cell events were collected using FACS Canto II (BD Bioscience).
- The percentage of 2-NBDG uptake was calculated from mean fluorescence intensity (MFI) compared with the control.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Choksi, A. and Chattopadhyay, S. (2022). Glucose (2-NBDG) uptake assay. Bio-protocol Preprint. bio-protocol.org/prep1948.
2. Choksi, A., Parulekar, A., Pant, R., Shah, V. K., Nimma, R., Firmal, P., Singh, S., Kundu, G. C., Shukla, S. and Chattopadhyay, S.(2021). Tumor suppressor SMAR1 regulates PKM alternative splicing by HDAC6-mediated deacetylation of PTBP1. Cancer & Metabolism 0(0). DOI: 10.1186/s40170-021-00252-x